PATENT COOPERATION TREATY

ATIONAL PRELIMINARY EXAMINING AUTHORITY

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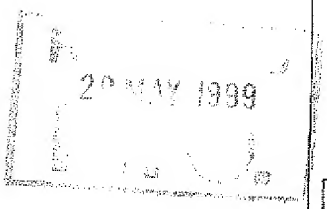

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Applicant's or agent's file reference PBA/D088187PWO	Date of mailing *(day/month/year)* 18.05.99
	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/00461	13/02/1998	13/02/1997

Applicant
THERAMARK LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PBA/D088187PWO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/00461	13/02/1998	13/02/1997
International Patent Classification (IPC) or national classification and IPC A61K47/48		
Applicant THERAMARK LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22/07/1998	18.05.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Giacobbe, S Telephone No. (+49-89) 2399 8463

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00461

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-39	as originally filed				

Claims, No.:

2-17	as originally filed				
1,18-25	as received on	16/04/1999	with letter of	15/04/1999	

Drawings, sheets:

1/1	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-25
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-25
Industrial applicability (IA)	Yes:	Claims	1-25 (cf Section 1.4)
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1- Sections V and VI

1.1 Documents cited

The following documents (D) are referred to in this Report:

D1 Chemical Abstract, 1990, vol 112(11), AN 90:91313

D2 US 5 387 692

D3 J. Med. Chem., 1991, 34, 2933-2935

D4 Proceedings of the American Association for Cancer Research Annual Meeting, San Diego, CA, April 12-16, 1997, see no 2894

D5 Anti-Cancer Drug Design, 1995, 10, 227-241

D6 British Journal of Cancer, 1995, 72, 1462-1468

D7 Database Dissabs, 1987, vol 49(3B), p. 745, AN 87:31004

D8 The Merck Index, 1989, item 4369

Document D8 was not cited in the International Search Report, but was cited from the examiner's own knowledge. (cf. Section VI).

1.2 Novelty (Art 33(2) PCT)

The present application does meet the requirements of Article 33(2) PCT, because the subject-matter of claims 1-25 is new.

The available prior art does not disclose any conjugate between a therapeutic agent and a bioreductive moiety which, upon bioreduction, is broken into its components, the bioreductive moiety being transformed into a species possessing a free (claim 1) or sterically unaccessible (claim 18) alkylating centre.

1.3 Inventive Step (Art 33(3) PCT)

The present application does not meet the requirements of Article 33(3) PCT, because the subject-matter being disclosed does not involve an inventive step.

Documents D3, D4 and D7, which are considered to represent the most relevant state of the art, disclose a method for targeted drug delivery to hypoxic solid tumours based on the principle of bioreductive prodrugs. The two products of the bioreductive brakedown of the described prodrugs are the biologically active substance and a lactone (in D4 and D7) or an aminoquinoline salt (in D3). In these documents, no

mention is made of tests performed *in vivo*. The subject-matter of the present application differs in the structural features of the by-product of the bioreductive activation of the active substance, thereby supposedly resulting in a carrier which has substantially no cytotoxic activity *in vivo* (cf. page 4, line 32 to page 5, line 9 of the description). In this context, the technical problem which the present invention sought to solve may be regarded as "*how to provide a non-cytotoxic type of carrier to be used in a method for targeted drug delivery to hypoxic tissues*". However, there is no evidence in the description showing that it has actually been solved, since no comparative toxicity studies are reported. Therefore the technical problem must be reformulated as "*how to provide a different type of carrier to be used in a method for targeted drug delivery to hypoxic tissues*". The solutions proposed in the present application (cf. independent claims 1 and 18) cannot be considered as involving an inventive step (Article 33(3) PCT) because they are merely one of several straightforward possibilities from which the skilled person would have selected without the exercise of inventive skills.

1.4 Industrial applicability (Art 33(4) PCT)

For the assessment of the present claim 23 on the question of whether it is industrially applicable, no unified criteria exist in the PCT, since the patentability of claims directed to further medical uses is *inter alia* dependent upon their formulation as well as upon national and regional laws. Furthermore claims 24 and 25, which are directed to a method of treatment of the human or animal body, may not be allowable under some patent systems.

2- Section VIII

2.1 It is clear from the description on page 4, line 22 to page 5, line 9 that it is essential for the definition of the invention that the bioreductive moiety, after delivery of the therapeutic agent, either should be itself a non-toxic molecule (cf. e.g. claim 18) or should be converted into a non-toxic molecule by an internal alkylation reaction. Newly filed claim 1 however does not unconditionally contain this last feature, since the expression "*being capable of...*" is not sufficient to introduce it, given that it does not mean that the internal alkylation reaction must in any case occur. Therefore, independent claim 1 does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

2.2 The embodiment of the invention described in example 8 (cf. page 39) does not fall

within the scope of the claims, since the composition contains no conjugate between a drug and a bioreductive carrier. This inconsistency between the claims and the description leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).

CLAIMS

1. A bioreductive conjugate comprising a non-cytotoxic bioreductive moiety with linked thereto at least one therapeutic agent, and salts thereof, said conjugate being such that on bioreduction the therapeutic agent is released with generation of a species having an alkylating centre and being capable of undergoing a self-alkylation reaction to generate a non-cytotoxic residue of the bioreductive moiety.

2. A bioreductive conjugate as claimed in claim 1 of formula I:

$$A\,(B)_n \qquad (I)$$

(where A is a non-cytotoxic bioreductive moiety, each B is independently the residue of a therapeutic agent, and n is an integer) or a salt thereof.

3. A bioreductive conjugate as claimed in claim 2, wherein in formula I, n is 1 to 3.

4. A bioreductive conjugate as claimed in claim 2 or claim 3, wherein A and B are stably conjugated in an oxygenated environment and are such that following reductive activation of A, A and B detach and either A is itself a stable, non-cytotoxic species, or A reacts with itself to form a stable, non-cytotoxic species.

5. A bioreductive conjugate as claimed in any one of claims 1 to 4, wherein said bioreductive moiety is substantially non-mutagenic.

6. A bioreductive conjugate as claimed in claim 1 of the formula II:

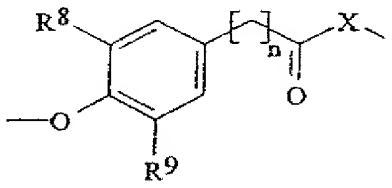
1,4-benzoquinone and the therapeutic agent is dexamethasone.

16. A bioreductive conjugate as claimed in any preceding claim, wherein said bioreductive moiety is linked to said therapeutic agent via a linker group L comprising an ester, phosphate ester, ether, amine, thiol or thiol ester group or any combination thereof.

17. A bioreductive conjugate as claimed in claim 15 wherein said linker group L is a group of the formula:

$$-O-CO-(CH_2)_n-CO-X-$$

or



(wherein n is an integer from 1 to 3;

X represents a sulphur or oxygen atom; and

R^8 and R^9 each independently represent F or Cl).

18. A bioreductive conjugate comprising a non-cytotoxic bioreductive noiety with linked thereto at least one therapeutic agent, and salts thereof, said conjugate being such that on bioreduction the therapeutic agent is released with generation of a species having a sterically hindered alkylating centre to prevent alkylation of biomolecules.

19. A process for the preparation of a bioreductive conjugate as claimed in any of claims 1 to 18, said process comprising linking at least one therapeutic agent to a non-cytotoxic bioreductive moiety.

20. A pharmaceutical composition comprising a bioreductive conjugate as claimed in any one of claims to to 18, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

21. A bioreductive conjugate as claimed in any one of claims 1 to 18 for use in a method of targeting a therapeutic agent to a site of hypoxia and/or ischemia within the human or non-human animal body.

22. A bioreductive conjugate as claimed in any one of claims 1 to 18 for use in treatment of rheumatoid arthritis or other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, Alzheimer's disease and other neurological disorders, cancer, kidney disease, digestive diseases, liver disease, chronic periodontitis or ischemia following tissue transplantation.

23. Use of a bioreductive conjugate as claimed in any one of claims 1 to 18 in the manufacture of a medicament for use as a targeting agent capable of targeting a site of hypoxia and/or ischemia within the human or non-human animal body.

24. Use as claimed in claim 22 for the treatment of rheumatoid arthritis or other arthritic conditions, diabetes, atherosclerosis, stroke, sepsis, alzheimer's disease and

other neurological disorders, cancer, kidney disease, digestive diseases, liver disease chronic periodontitis or ischemia following tissue transplantation.

25. A method of targeting hypoxic and/or ischemic tissues in the human or non-human animal body, said method comprising administering to said body a bioreductive conjugate as claimed in any one of claims 1 to 18.